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National Toxicology Program, Report on Carcinogens
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Re: Comments on the Nomination of aristolochia-related herbal remedies
And Aristolochic acid for Listing in the NTP Report on Carcinogens,
12th
Edition (June, 2004).

Dear Dr. Jameson:

The National Institute of Toxicological Research (NITR) is a national organization for toxicological testing and research under the Korea Food and Drug Administration (KFDA). The Institute strives primarily to understand important biological triggering mechanism and improves assessment methods of human exposure, sensitivities, and risk by conducting basic, applied, and policy research on the adverse effects of the regulated products such as foods, food additives, and drugs.

Our organization has been operating the Korea National Toxicology Program (KNTP) from 2002. The KNTP has constantly discussed with US NTP in order to ensure the KNTP is well established. The two groups share common interests in the fields of biomedical research and related training relevant to the work of their respective national toxicology program.

As position of partner, we would like to share our data of aristolochia-related herbal remedies and Aristolochic acid for Listing in the NTP Report on Carcinogens, 12th Edition (June, 2004). KNTP can serves as a public comment on relevant information concerning their toxicity, use patterns, human exposure information.

I am the principal investigator of 90-day repeated toxicity study and genotoxicity about aristolochia-related herbal remedies such as *Aristolochiae debilis* and *Aristolochiae contort* and aristolochic acid. These studies are funded by KFDA, and the objectives of these studies are to evaluate the safety of *A. contorta* or *A. debilis* in Korea Traditional Medicine; they were completed in 2003 and peer reviewed histopathology data in 2004.

Now we are translating the report into English version.

This is the first time to attend the public comment in the RoC, and we want to participate in exchanging of scientific information.

Please do not hesitate to contact me if you need any additional information.

Yours sincerely,

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Safety assessment of domestic herbal medicine (*Aristolochiae Radix*)

Abstract

Aristolochiae fanchi (*A. fanchi*) has been used in herbal medicines, especially in China. However, Chinese young woman who had been placed on weight loss program including administration of *A. fanchi* extracts developed kidney damage, nephropathy, and neoplasm of bladder. *A. fanchi* has been shown to include a toxic substance called aristolochic acid (AA) which is known to be nephrotoxic, carcinogenic and mutagenic. Recently, the US Food and Drug Administration (FDA) found that 18 of products that may have been sold as traditional medicines or as ingredients in dietary supplements contained AA. Based on these analytical results, FDA is advising consumers to immediately discontinue use of any botanical products containing AA. Roots and fruits of *Aristolochia* containing AA have been used for various therapeutic purpose in Chinese traditional medicine. *Aristolochiae fructus* has been used to decrease blood pressure and in treatment of cough, excecration, asthma and hemorrhoids while *Aristolochiae Radix* has been used for treatment of enteritis, dysentery, swelling and abdominal sidtention. Although natural medicinal products of *Aristolochia concorta* (*A. concorta*) come from whole country except Je-ju island in Korea, the imports from China is now increasing. Therefore, this study were performed to evaluate acute and sbuchronic toxicity, and genotoxicity of domestic *A. concorta*.

A. concorta used in this study were collected in the area of Daeam mountain (Yang-goo Gun, Gwang-won Do), freeze-dried and then used after certification as domestics by Department of Oriental Medical Science of Kyunghee University. Freeze-dried products of *A. fructus* contain 2.29 ug/mg of AA while those of *A. Radix* contain 2.72 ug/mg of AA.

Genotoxicity

To assess the genotoxicity of dried powder from *A. fructus* and *A. Radix*, several

classical toxicological tests were performed. In Ames test, both test items did show transformation of revertant both with or without S9 metabolic activating system in TA100, TA98, TA1537 and E. coli WP2 uvrA, indicating the mutagenic effect of the test items. To assess clastogenic effect, *in vivo* micronucleus assay were also performed using male ICR mice. *A. fructus* and *A. Radix* were suspended in sterile distilled water and orally administered twice at 24 hr intervals at doses of 0, 12.5, 25, and 50 mg/kg, and 0, 6.25, 12.5, and 25 mg/kg of AA, respectively. Both test items caused weak but dose-related increase in the incidence of micronucleated polychromatic erythrocytes at the dose levels tested, suggesting weak clastogenicity *in vivo*. Therefore, we conclude that both of *A. fructus* and *A. Radix* have mutagenic effect in our test system.

General toxicity

To evaluate the acute toxicity of dry powder form *A. Radix* by a single oral administration, the test item was orally administered to two groups containing two rats at dose levels of 2,000 and 5,000 mg/kg body weight. No treatment-related deaths were found in two groups during the 14-day observation period, but they showed salivation and soiled perineal region. We were trying to ascertain the toxicity profile of test item in 2-week administration period. The test item was orally administered to groups of rats containing five males and females at dose levels of 31.3, 125, 500, 2,000, 5,000 mg/kg body weight. Clinical signs observed in 2,000 and 5,000 mg/kg males and females were salivation and soiled perineal region. A statistically significant decrease in body weight was observed in 5,000 mg/kg male group. Gross findings at necropsy observed in the present study were shown in thickening of stomach and atrophy of prostate and seminal vesicle. Histopathological findings observed in the present study were shown in stomach. Both sex of more than 500 mg/kg groups exhibited squamous cell hyperplasia with hyperkeratosis in the nonglandular stomach. These findings indicated that the test item of more than 500 mg/kg (1.36 AA mg/kg) might affect histopathologically

forestomach.

Thus, 13-week orally repeated dose toxicity was investigated to ascertain the toxic effects of *A. radix* in F344 rats at dose levels of 0, 1 (0.003 AA mg/kg), 5 (0.014 AA mg/kg), 25 (0.068 AA mg/kg), 125 (0.34 AA mg/kg), 500 mg/kg (AA 1.36 mg/kg). No mortalities were found in any of the dose groups including vehicle control groups of both sexes during the study period. Hematologic and serum biochemical examinations revealed no changes related to the test item in any of the dose groups of both sexes. However, gross findings at necropsy implicated thickening of the forestomach wall. In histopathological examinations, prominent findings related to the test item treatment were observed in the forestomach and urinary bladder. There were squamous cell papilloma, squamous cell hyperplasia, ulceration and erosion observed in the non-glandular stomach. Squamous cell hyperplasia was observed at dose levels of more than 125 mg/kg in both sexes and squamous cell papilloma was observed at dose level of 500 mg/kg in both sexes. The incidence and severity of these proliferating lesions including squamous cell hyperplasia and squamous cell papilloma increased with dose dependency. Transitional cell hyperplasia was also observed in the urinary bladder at dose levels of more than 25 mg/kg in both sexes and the incidence and severity of the lesion increased with dose dependency.

In conclusion, the toxic changes related to the test item treatment were observed in the stomach and urinary bladder, and the NOAEL was estimated to be 5 mg/kg/day for both males and females in F344 rats.

Key words : *Aristolochiae radix*, Aristolochic Acid, Stomach, Urinary Bladder, F344 rats